TAR CANCER IN MICE. II: THE CONDITION OF THE SKIN WHEN MODIFIED BY EXTERNAL TREATMENT OR DIET, AS A FACTOR IN INFLUENCING THE CANCEROUS REACTION.

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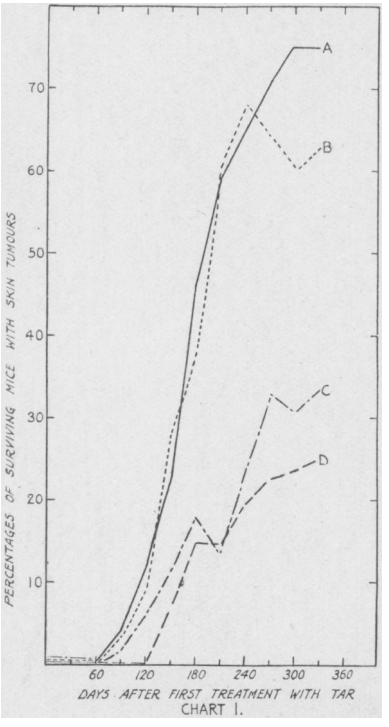
THE present paper is concerned with a consideration of the effect of the local and general condition of the skin of mice, as modified by external treatment or diet, on the carcinogenic activity of tar.

METHODS.

With few exceptions, the general methods outlined in a previous paper (Watson and Mellanby, 1930) were employed. The technique used for experimentally modifying the local or general condition of the skin is described at the beginning of each section concerned. Unless otherwise stated the animals have been maintained on the control diet, consisting of powdered bread (4 parts), Sussex ground oats (1 part) and minced cabbage (1 part), mixed in an electric mixer and made into a uniformly moist paste with water; this diet was fed daily and supplemented once per week with cod-liver oil 0·25 c.c. per mouse and marmite 0·25 gr. per mouse.

The standard method of applying the tar to the epilated skin of the animal has been outlined in the previous paper, although in comparative experiments it has been found advisable under certain circumstances to reduce the severity of the tar treatment of both experimental and control animals in order to magnify any differences that may possibly exist in the reaction of the mice to the experimental conditions. Loeb (1924) and Slye (1925) are both of the opinion, for instance, that if an irritant is strong enough it may overwhelm and conceal any inherited resistance to the development of cancer. Similarly, of course, it might be anticipated that any resistance induced by a particular experimental treatment would be masked if the conditions for inducing the tumours were too severe. Accordingly, certain sub-optimum conditions have been used on occasions in the present investigations. Possibly the easiest method of decreasing the severity of the treatment would be to use a diluted tar, although Hieger (1929) has shown that it is necessary to dilute an ether

extract of tar more than ten times before a significant decrease in the carcinogenic activity may be expected. If a diluent whose inertness in concentrations such as this were certain, could be found, such a method would probably be the most useful. In the present experiments, however, two other methods have been used. In some cases sub-optimum conditions have been produced by a modified tarring technique. This has consisted of the



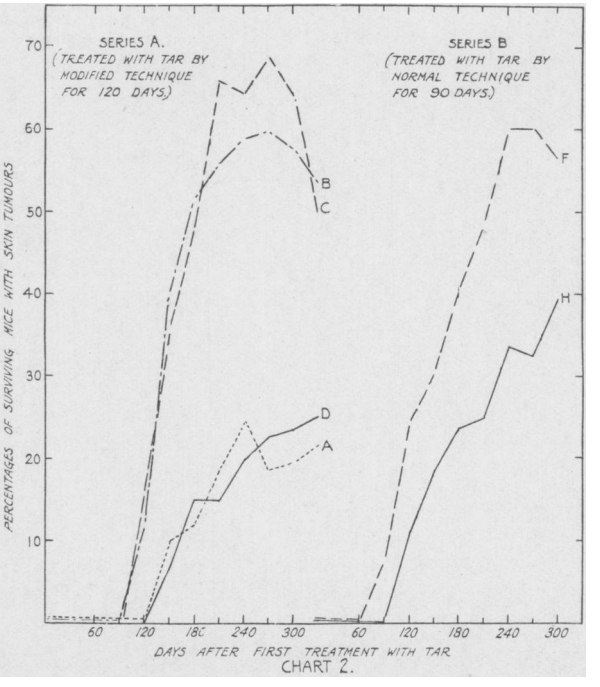


CHART 1.—Relative percentages of surviving animals with skin tumours in four groups of mice treated with tar by normal technique (curves A and B) and modified technique (curves C and D).

CHART 2.—The effect of treating skin of animals with (1) tannic acid (curve A), (2) petroleum ether extracts of mice (curves B and C), and (3) olive oil (curve F) before applications of tar. Curves D and H = control groups in series A and B respectively.

rapid treatment of the mice with the tip of a camel-hair brush, without removing the animals from the box. It was found that a quick operator could complete the treatment of a box of mice while the animals were still huddled together in one corner. The treated area was approximately half the size covered in the normal way by the ordinary technique, and this, together with the diminished pressure given to the tarred brush, produced the sub-optimum conditions, the results of which are shown in Chart 1.

Curves A, B, C and D show the relative percentages of animals surviving with skin-tumours in four groups of mice maintained under the same

conditions of diet, etc. Groups A and B were tarred by the normal technique twice weekly for 120 days and C and D by the modified technique for the same period. The experimental condition indices (E.C.I.*) were 25·3 and 22·5 for the two groups tarred by the normal technique and 10·0 and 7·0 for those tarred by the modified technique. Considering the difficulties of this type of prolonged experiment, a fairly satisfactory agreement exists between the two series of results, especially in view of the fact that the four groups of mice were tarred during different periods and did not form part of any one experiment, although of course, the same tar was used in all cases. In all experiments a control group has been included in each series, as even when care is taken to eliminate sources of error, the similar reaction of different groups of mice to apparently the same tarring technique cannot be relied on with more than a moderate degree of certainty. Another method of decreasing the severity of the treatment with tar has been used in other cases by employing the normal technique but limiting the treatment to 90 instead of 120 days. Bang (1922) showed that under these conditions a tar which induced 100% tumours in four months gave a 70% incidence only.

THE TREATMENT OF THE SKIN IN VARIOUS WAYS PRIOR TO THE TREATMENT WITH TAR.

Any local or other treatment of the skin which facilitates the absorption of the carcinogenic agent through the cells of the epidermis, should, *primâ facie*, speed up the malignant processes in one way or another. Such treatment has been tried by several workers more with the direct object of hastening the appearance of the tumours than of watching its effect on their subsequent development. Bartozek (1926), for instance, produced tumours in 68 days in mice whose skin was cleansed with ether before the application of tar, whereas in the untreated controls, 103 days elapsed before their appearance. Twort and Twort (1929) are of the opinion, on the other hand, that lanolin when applied to the skin in the intervals between synthetic tar applications may substantially delay the development of the tumours.

It seemed to us possible that the carcinogenic properties of tar may depend on its solubility in fats, and because of the presence in the skin of such a medium, that the tar can penetrate and gain access to the reacting cells. The addition of suitable fluid fats to the skin in amounts not too great to lower appreciably the dilution increases the cancerous reaction. The treatment of the skin with petroleum ether, a typical fat solvent, on the other hand, under certain conditions may preclude or at any rate hinder the skin fat functioning in this way. These facts which will be dealt with briefly, are shown most clearly if the effects of the tar are watched for extended periods after the last application of tar.

* The experimental condition index (E.C.I. value) of any particular group may be defined as the average of the percentage liabilities of the mice surviving without skin-tumours at the end of each arbitrarily chosen period during an experiment, to develop such tumours before the end of the next period. With the tar used in the present investigations and in an experiment lasting 240–480 days, 60 days has been selected as a suitable period. The details of the determination of these E.C.I. values have been described in a previous paper (Watson and Mellanby, 1930).

1. *The Local Treatment of the Skin with Tannic Acid and Certain Animal Extracts.*

The influence of certain fluid fats in facilitating the absorption of the tar and the failure of such a substance as tannic acid to produce any effect is illustrated in the following experiment. The most suitable fat to test in the first place seemed to be one closely allied to the skin fat of the animal. Such a fat was obtained by extracting the bodies of a number of mice after the removal of the alimentary tract with successive fresh batches of petroleum ether (B.P. 40°–60° C.) on a water-bath. After filtration the petroleum ether was removed by distillation, and the extract (a mixture of fats and fatty substances usually quite fluid at 70° F., the temperature of the animal room) used for the local treatment of the epilated skin of batches of mice before the treatment with tar. Four groups of mice (each group consisting of 70 animals) were maintained under similar conditions of diet, etc., for a period of 360 days. For the first 120 days the animals were treated twice weekly with tar by the modified technique described above. Thirty minutes before each application of tar throughout the experiment, the mice in each group were treated by means of a camel-hair brush at the site of tarring, as follows: The animals of group A were treated with a 10% and later in the experiment a saturated solution of tannic acid in water, those of group B with a petroleum-ether extract of mice which had died with tar tumours, and those of group C with a similar extract of normal mice. The animals of group D received the tar applications only and served as a control. Chart 2 (series A) shows the percentages of the surviving mice with skin-tumours during the experiment, Chart 3 the relative rates of growth of these tumours, and Chart 4 the numbers and size of the lung nodules (metastases to the skin-tumours or epithelial tumours of the lung) found in the skin-tumour bearing mice at the post-mortem examinations.

These three charts show clearly that the progress of malignancy has been accelerated by the application of the homologous fats to the skin before the tar treatment. The tannic acid solution on the other hand seems to have exerted no influence, the group showing a close resemblance in its behaviour to the control. The experimental condition indices were 334 and 418 for the two groups treated with the animal extracts and 116 for the group treated with tannic acid, as compared with 100 for the control group (Table I). The primary skin tumours in the two fat-treated groups attained in many cases an enormous size. Frequently they showed a tendency to originate at a number of different points of the epilated skin, with the result that a tumour in some cases developed from as many as ten small isolated papillomata. The numbers and size of the lung nodules in the mice of the four groups were also of interest. It will be seen from Table I that the percentages of the tumour-bearing animals with visible nodules of the lung were 47 and 50 for the tannic acid group and the control respectively. In the fat-treated groups the corresponding percentages were 63 and 59. The relative numbers and sizes of these nodules are shown in Chart 4. The development in the group treated with the extract of normal mice (group C) was of a higher order than has been met with in any group during the present investigations.

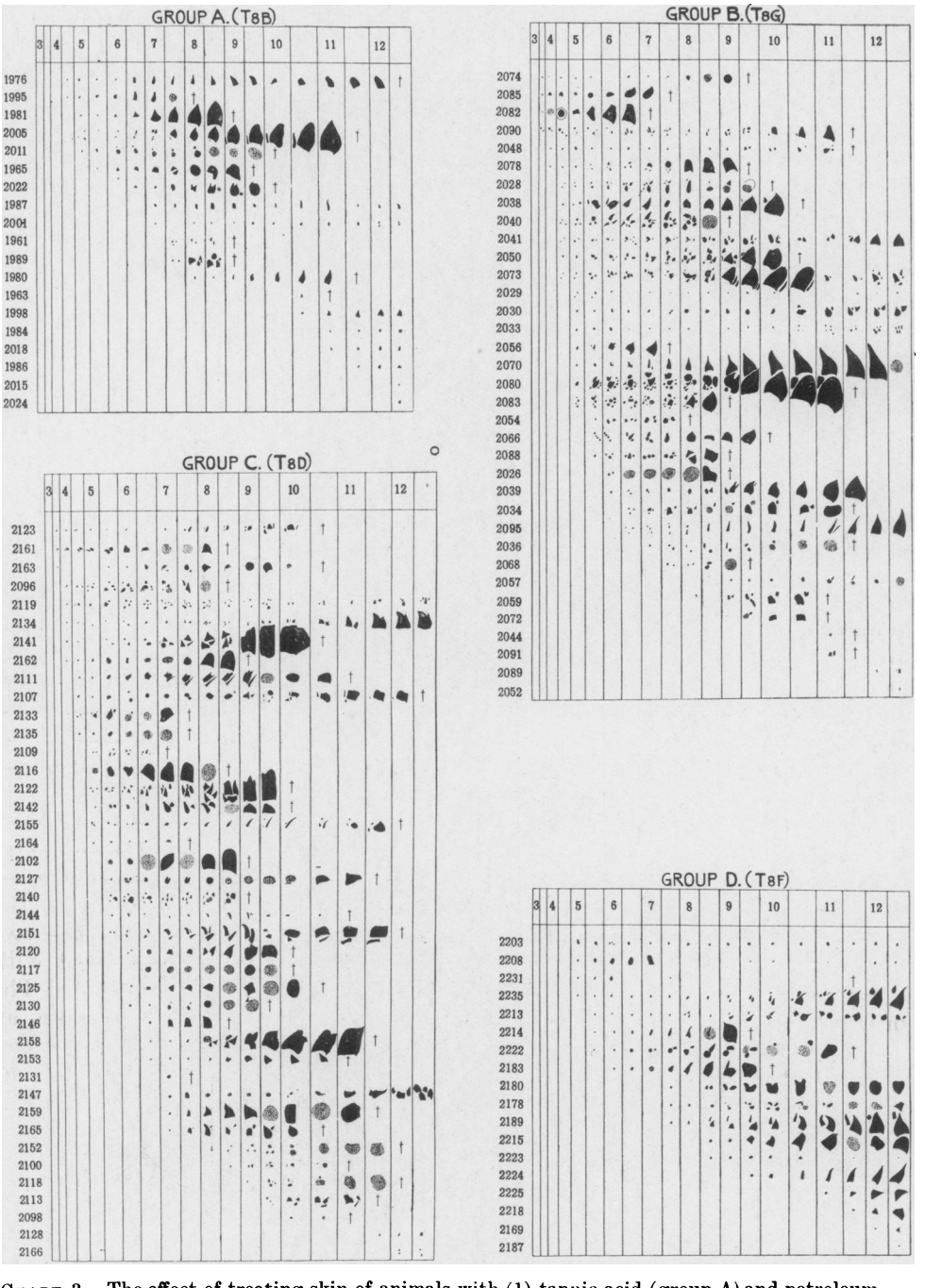


CHART 3.—The effect of treating skin of animals with (1) tannic acid (group A) and petroleum ether extracts of mice (groups B and C) before each application of tar, on the rate of growth of the skin tumours. Group D = control group (tar applications only). Tumours charted at 14 day intervals for 12½ months. Hæmorrhagic tumours shown by means of dotted areas. Scale $\frac{1}{10}$ natural size.

2. *The Local Treatment of the Skin with Olive Oil.*

The local treatment of the skin with olive oil thirty minutes before each of the bi-weekly applications of tar produces similar but less marked effects to the animal extracts employed above. Two groups each of 70 mice were treated with tar for 90 days, the animals of one group receiving local treatment with olive oil before the applications of tar, the other group receiving the tar treatment only. Chart 2 (series B) shows the percentages of the surviving mice

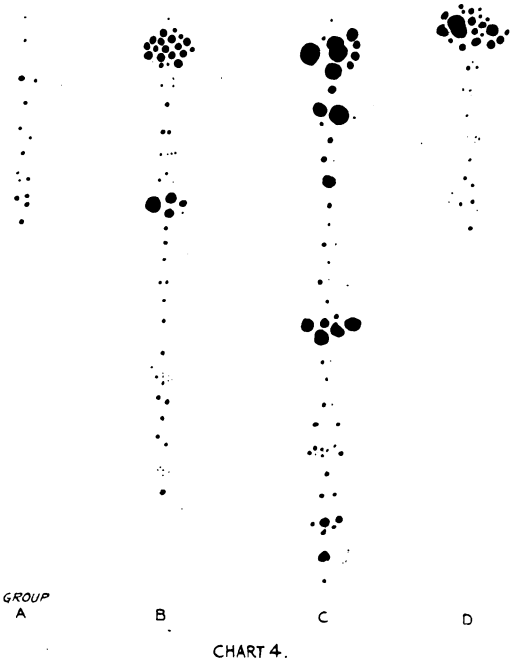


CHART 4.—The effect of treating the skin of the mice with tannic acid (group A) and petroleum ether extracts of mice (groups B and C) before the applications of tar, on the numbers and size of the lung nodules of the tumour-bearing animals. Group D was treated with tar only.

with skin tumours during the experiment in the two groups. The animals of the olive-oil treated group (curve F) showed a greater tendency to develop tumours than those of the untreated group (curve H). This is also illustrated by the experimental condition index, which was 175 in the oil-treated group compared with 100 in the control group (Table I). The rate of growth of the skin tumours was approximately the same in the two groups (charts not shown), but the percentages of the tumour-bearing animals with lung nodules was 72 in the oil-treated group compared with 57 in the untreated group (Table I). The tendency of the skin tumours to originate at a number of isolated centres scattered over the epilated area was again apparent as compared with the

control group, although to a less extent than in the case of the previous experiment, where the skin was treated with the animal fatty extracts.

It will be seen, therefore, that the application of fluid fats to the skin of the mouse before the periodical treatments with tar shows a tendency to accelerate the cancerous reaction. This is somewhat less obvious with such a fat as olive oil than with fats or fatty substances allied in their chemical make-up to the skin and body fats of the animals. The acceleration of the malignant processes

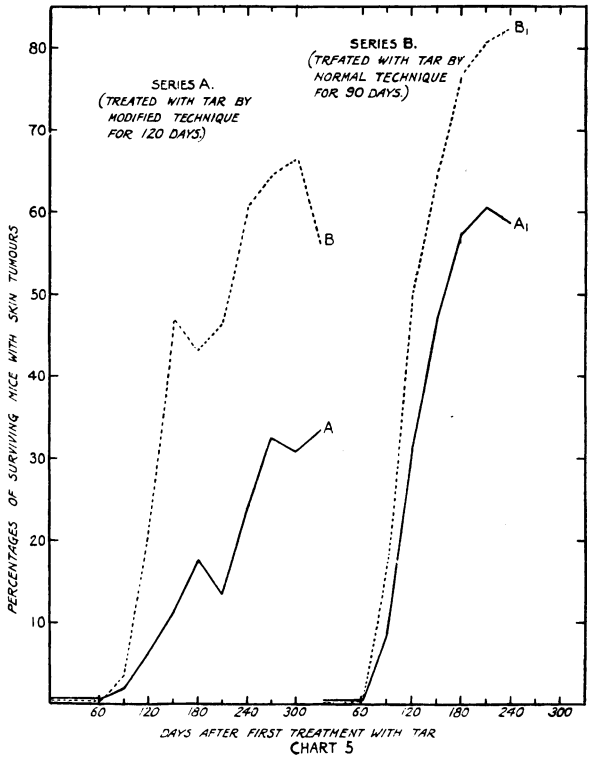


CHART 5.—The effect of mantaining the mice on a diet containing a high proportion of butter (curves B and B_1) on the reaction of the animals to the tar. Curves A and A_1 = control groups in each series.

is shown by increased liabilities of the treated mice to develop skin-tumours and nodules of the lung. There is also a tendency for the skin-tumours to be initiated at a number of isolated points on the treated area of the skin rather than at a single point, which is typical, with the tar used in the present experiments, of the mice treated with tar only.

Since the local treatment with fluid fats produced these accelerated effects, it appeared of interest to see whether a similar acceleration could be produced by feeding the animals with diets containing a high proportion of fat during the

experimental period. It has been observed by Boyd and Roy (1929) that mice fed on diets containing vegetable ghee tend to assume a very characteristic appearance, the hair becoming straight and greasy and the animals appearing as if they had been dipped in fat. Moreover, it is known that the composition of the body-fats can to a large extent be controlled by the type of fats with which the animal is fed. Accordingly, groups of mice were maintained

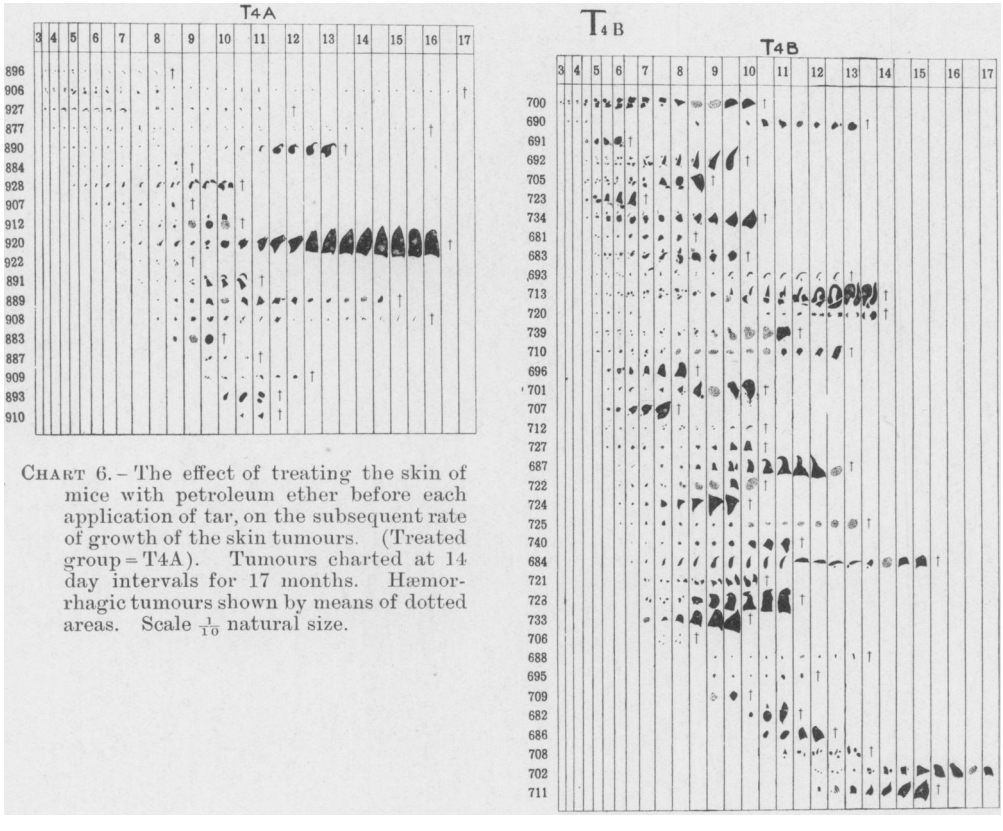


CHART 6.—The effect of treating the skin of mice with petroleum ether before each application of tar, on the subsequent rate of growth of the skin tumours. (Treated group = T4A). Tumours charted at 14 day intervals for 17 months. Hæmorrhagic tumours shown by means of dotted areas. Scale $\frac{1}{10}$ natural size.

on diets containing a high proportion of fat in the form of butter, the carcinogenic activities of the tar being watched over a long period, and the effects compared with those produced in groups of mice maintained on the control diet. These experiments will be briefly described.

3. *The Effect of Diets containing a High Proportion of Butter on the Carcinogenic Activities of the Tar.*

Two groups each of 70 mice were treated twice weekly with coal-tar by the modified tarring technique described above for a period of 120 days, and the development of tumours watched over a further period of 330 days. During this

time group A received the control diet (powdered bread 4 parts, Sussex ground oats 1 part, mixed in an electric mixer and made into a uniformly moist paste with water; diet fed daily, and supplemented once per week with cod-liver oil and marmite). Group B received the same diet with added butter in amounts varying from 12·5 to 25% during the experiment. Under these circumstances the animals showed the characteristic coat effect described by Boyd and Roy (*loc. cit.*). This was particularly obvious during the first few months of feeding the butter diet, although as the experiment progressed the effect became less marked. It is probable that the absorption of the tar was facilitated by the general condition of the skin which had been induced through the medium of the diet. Correlated with this there was a general acceleration of the carcinogenic activities of the tar. This is shown by the increased numbers of animals with tumours throughout the experiment (Chart 5, series A) and an increased experimental condition index (244 compared with 100 for the control group). Further, 60% of the tumour-bearing animals in the butter-fed group contained lung nodules compared with 37% in the control group (Table I).

This general effect of the acceleration of the carcinogenic activities of the tar when applied to mice fed on diets containing a high proportion of butter receives support from a further experiment, the curves for which are included in Chart 5 (series B). The experimental group mice received the same diet as the control, with added butter in amounts varying from 19 to 25% during the experiment. Sub-optimum conditions were produced by limiting the normal treatment with tar to 90 days. The curves in the chart show the acceleration of the carcinogenic activities in the case of the butter-fed mice (curve B 1) compared with the control group (curve A 1), and confirmation is obtained from the increased experimental condition index (157 compared with 100) and increased incidence of lung nodules (55% compared with 41%) shown in Table I.

Evidence has been presented, therefore, in support of the hypothesis that the carcinogenic action of tar is dependent to some extent on its fat-soluble characteristics when applied to the skin of the mouse. The carcinogenic activities can be increased, for instance, by the local treatment of the skin with suitable fluid fats or fatty substances (such as a petroleum-ether extract of the tissues of mice), or olive oil, before each of the periodical treatments with tar. They can also be accelerated by so modifying the general condition of the skin by such means as maintaining the animals on a high butter diet that the absorption of the tar is facilitated. This, in effect, produces similar results to the purely local application of fluid fats, except that if the conditions favourable to the growth of the mice are in any way impaired, the acceleration of the cancerous reaction is shown more in the numbers of tumours produced than in their subsequent growth. (One result of this is shown in the comparatively small size of the lung-nodules of the butter-fed mice compared with those of the control group after the same period of time).

If the tar then gains access to the reacting skin-cells in this way, it might be anticipated that since the artificial provision of adequate transport facilities accelerates the cancerous reaction in the manner described above, the removal, even in part, of the fatty substances of the skin would exert the opposite effect, with a resulting slowing down of the carcinogenic processes.

There is, however, a lack of agreement in the literature on the effects of treating the skin with such a fat solvent as xylol or petroleum-ether before the periodical applications of tar. The results obtained, of course, might depend on the conditions employed, since in addition to dissolving fats, these solvents probably dissolve part at least of the carcinogenic substances of the tar. If, therefore, the conditions of treatment with one of these solvents are such as to remove at any rate a part of the superficial skin fatty substances and leave no trace of the solvent, it might be expected that the absorption of the tar, if applied immediately afterwards, would be delayed. If this is repeated before every application of tar, a substantial slowing-up of the cancerous reaction might be anticipated. If, on the other hand, the treatment with petroleum-ether is such as in no way to interfere with the skin fat, but merely to effect the solution of the carcinogenic active fraction of the tar and mechanically facilitate its spread into the tissues of the epilated area, then the opposite effect—the acceleration of the cancerous reaction—might be expected. Such a condition might exist if the tar were applied immediately after the solvent and before the latter had evaporated away. Although this latter possibility has not been tested, the general conception receives some support from the following experiment.

4. *The Local Treatment of the Skin with Petroleum-Ether.*

Two groups, each of sixty male mice, were maintained under standard conditions of diet, etc., for a period of 480 days. The mice of one group (T. 4A) were treated with petroleum-ether (B.P. 60°–80° C.). This was applied to the previously epilated area by means of a cotton-wool plug as thoroughly as possible in order to remove, at any rate in part, the fatty substances present in the skin. Thirty minutes later, when the animals had recovered from the effects of the treatment and no traces of the solvent remained on the skin, the animals were tarred by the normal technique (*i. e.* under the optimum conditions). This treatment was continued twice weekly for 120 days and the mice were kept under close examination for a further period of 360 days. The animals in the control group (T. 4B) received the routine twice weekly application of tar without any preliminary treatment of the skin with petroleum-ether. The number of tumours was relatively less in the petroleum-ether treated group (experimental condition index 72 compared with 100 for the control group), and the difference between the two series is shown clearly in the progress of the tumours after the cessation of the tar treatment (Chart 6).

Prior to the ninth month the tumours in the treated group for the most part appeared papillomatous in type, and quite insignificant in size compared with those of the untreated group. After this time the rate of growth became slightly more accelerated in the treated group, but with one exception (No. 920) where the tumour grew on the extreme edge of the treated area, the development of the tumours of the experimental group was poor compared with that of the control group. Further, the lungs of only 47% of the tumour-bearing animals contained visible nodules as compared with 61% in the control group mice (Table I).

TABLE I.—*Details of Experimental Results.*

Experiment number.	Number of mice.	Treatment of skin before tarring.	Period of experiment.	Total number of tumour-bearing mice.	Group experimental condition index (E.C.I.).*	Number of tumour-bearing mice examined.	Percentage of tumour-bearing mice with lung nodules.
T. 8B	70	Tannic acid	360 days	19	116	19	47
T. 8C	70	Animal Ext. I	360 „	35	334	35	63
T. 8D	70	Animal Ext. II	360 „	41	418	41	59
T. 8F	70	Untreated control	360 „	18	100	18	50
D. 13D	70	Olive oil	300 „	29	175	29	72
D. 13C	70	Untreated control	300 „	21	100	21	57
T. 4A	60	Petroleum-ether	480 „	19	72	19	47
T. 4B	60	Untreated control	480 „	37	100	36	61

In the following two cases a similar condition of the skin to that induced by the local treatment with suitable fluid fats was brought about by maintaining the mice on a diet in which an increased amount of fat, in the form of butter, was included:

D. 5B	70	Butter-fed	450 days	39	244	38	60
D. 5L	70	Control	450 „	20	100	19	37
D. 17B	90	Butter-fed	240 „	31	157	31	55
D. 17D	90	Control	240 „	37	100	37	41

* Referred to value of 100 for control group in each series.

SUMMARY.

1. The carcinogenic activities of a sample of coal-tar were increased when each of its periodical applications to the skin of the mouse was preceded by treatment of the skin with certain fluid fats or fatty substances. A petroleum-ether extract of the tissues of mice was found to be particularly effective in producing this acceleration.

2. In addition to a relatively larger number of skin-tumours and their accelerated growth, these increased carcinogenic activities were reflected in the greater number and development of the associated nodules situated in the lung tissues.

3. The carcinogenic activities of the tar were also increased if the experimental mice were maintained on a diet containing a high proportion of fat in the form of butter. Under these conditions the coats of the animals assumed a characteristic fatty condition which probably facilitated the periodical absorption of the tar.

4. The preliminary treatment of the skin with petroleum-ether under certain conditions was found to produce the opposite effect to that produced by the local treatment of the skin with suitable fluid fats. This was shown by a decreased number of skin-tumours and lung-nodules, together with inhibited growth in the former.

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One of us (A.F.W.) also wishes to express his thanks to the University of London for the Laura de Saliceto Research Studentship, which was held during the course of the work.

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THE THEORETICAL CARBON DIOXIDE DISSOCIATION CURVE IN ACID-BASE DISTURBANCE OF CHILDHOOD.

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THE capacity of the blood for holding carbon dioxide depends upon two factors, which may be briefly described as the available alkali and the degree of buffering. The relative importance of these two factors in maintaining acid-base equilibrium is revealed by the theoretical dissociation curve described by Barcroft, Dryerre, Meakins, Parsons and Parsons. Such a curve may be constructed if the carbon dioxide content of a sample of blood be known for each of two tensions of carbon dioxide together with the cH of the blood at one of these tensions. The theoretical curve differs from that obtained in the usual way only at tensions of carbon dioxide below 10 mm. Hg., in that it cuts the y-axis some distance above the origin, which distance represents the volumes per cent. of combined carbon dioxide that would be present were it not for the action of the released hæmoglobin. The theoretical amount of combined carbon dioxide at zero tension is thus really an expression of the amount of base left over after the affinities of all the other (non-gaseous) acid radicles are satisfied, *i. e.*, the minimal amount of base available for combination with carbon dioxide. The theoretical curve also affords information as to the extra amount of carbon dioxide which can be taken up by the blood as a result of unit increase of cH ($cH \times 10^8$).

The results here recorded were obtained during the course of an investigation into disturbance of acid-base equilibrium in childhood. Several